Effects of Some Spices on Acid Production by Starter Cultures

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ABSTRACT

Ginger, red pepper, mustard, mace, cinnamon and clove were examined to determine their effects on growth of and acid production by a starter culture containing *Lactobacillus plantarum* and *Pediococcus cerevisiae* in a liquid medium. At 4, 8, and 12 g/l levels all spices except clove stimulated acid production by the starter bacteria but did not stimulate increases in bacterial population. Clove was inhibitory to the starter bacteria at and above the 4 g/l level, but low concentrations (0.5 - 2.0 g/l) stimulated acid production. High concentrations of cinnamon (8 and 12 g/l) delayed acid production, but bacterial counts were similar to those of the control.

The antimicrobial properties of spices have been investigated for many years (*5-7,15,21*). Most of the published reports deal with the effect of essential oils of spices and other plant materials on a variety of microorganisms (*2,4,16-18*). Koedam (*14*) reviewed the literature on antimicrobial action of essential oils for the period 1960-1976. Reviews of earlier work on spices may be found in references *4, 7,* and *20*. Considerable variation in resistance of different microorganisms to a given spice and of the same organism to different spices has been observed (*4,7*). Although much attention has been paid to pathogenic microorganisms (*4,6,9,13,20*), little information is available on the effect of spices on nonpathogenic microorganisms, particularly those used as starter cultures in the food industry. Recently Salzer et al. (*19*) reported on the effect of black pepper and its constituents on several species of starter culture organisms and fecal bacteria. *Lactobacillus plantarum* was inhibited the least, *Micrococcus specialis* and *Streptococcus faecalis* were somewhat inhibited and *Escherichia coli* was inhibited the most. These workers showed that fermented sausages can be prepared with encapsulated pepper extract when starter cultures are used. Karaioannoglou et al. (*11*) found that garlic is inhibitory to *L. plantarum* at concentrations greater than 1%.

Our studies on Lebanon bologna (*23*) indicate that addition of spices to the sausage formulation enhanced acid production during fermentation by either the natural microflora present in ground meat or by added starter culture. We employed Lactacel MC starter culture composed of *L. plantarum* and *Pediococcus cerevisiae*. Recently we reported on the effect of Lebanon bologna spice mixture and its major components, black pepper, allspice, and nutmeg, on growth of and acid production by Lactacel MC starter culture in a liquid medium (*12*). These spices stimulated acid production by the starter culture; however, this effect could not be attributed to increased bacterial population, since the bacterial counts of spice-containing samples (4, 8 or 12 g/l) did not differ significantly from those of the controls.

This paper reports on the effect of the other spices used in our Lebanon bologna formulation, red pepper, clove, cinnamon, ginger, mustard and mace, on starter culture Lactacel MC in a liquid medium.

EXPERIMENTAL

Spices

Sterilized red pepper, clove, cinnamon, ginger, mustard and mace (Griffith Laboratories[3], Inc., Union, NJ) were used throughout the experiment.

Liquid medium

Beef extract (Difco Labs, Detroit, Mich.), 3 g; tryptone (Difco), 5 g; sucrose, 20 g; and glucose, 20 g were dissolved in 1 liter of distilled water. The pH of the solution was adjusted to 6.4 with 0.1 N H_2SO_4 to give a post-sterilization pH 5.8-6.1. Aliquots of 250 ml of the medium were dispensed into 500-ml Erlenmeyer flasks and sterilized for 15 min at 15 psi.

Starter culture

Lactacel MC (Merck and and Co., Inc., Rahway, NJ) containing *L. plantarum* and *P. cerevisiae* was used in our fermentation work. In some experiments the individual organisms were used: *P cerevisiae* (Lactacel, Merck and Co.) and *L. plantarum* (Lactacel DS, Merck and Co.).

Fermentation

Sterilized spices were added aseptically to the flasks of sterile medium to provide concentrations of 0.5, 1, 2, 4, 8, or 12 g/l, respectively. Then 2.5 ml of commercial starter culture diluted with 0.5% peptone water was added to each flask and to a control containing no spice to give an initial bacterial population in the range of $1.0\text{-}5.0 \times 10^6$ cells/ml. The flasks were incubated statically for 4 days at 35 C. Samples for bacterial counts and titratable acidity were taken at 24-h intervals.

Bacterial counts

Bacterial counts were made by conventional pour plate techniques with tryptone glucose extract agar (Difco). Plates were incubated for 48 h at 35 C.

Titratable acidity

Titratable acidity was expressed as ml of 0.1 N NaOH required to titrate to pH 7.0 a 10-ml aliquot of the liquid medium after

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[2]*Agricultural Research, Science and Education Administration, U.S. Department of Agriculture.*

[3]*Reference to brand or firm name does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.*

centrifugation and dilution with 50 ml of distilled water. The initial titratable acidities of the liquid media were 0.54-0.75 ml. Addition of all spices except clove did not change the initial value significantly.

RESULTS AND DISCUSSION

The spice concentrations used in this work were chosen to encompass the levels used in Lebanon bologna formulations. The higher concentrations were used to determine possible germicidal effects of spices.

All the spices examined enhanced acid production by Lactacel MC starter culture but did not stimulate bacterial growth. The effect of spice concentration and the extent of stimulation of acid production was characteristic for each spice studied. The amount of acid produced, expressed as titratable acidity, and the bacterial counts for cultures exposed to 4, 8, and 12 g/l of ginger, mace, mustard, red pepper, and cinnamon were measured at 24-h intervals for 4 days as shown in Fig. 1-5, respectively. Bacterial counts for both the control and the spice-containing samples increased from 10^4 to 10^8 cells/ml after 24 h and remained in that range throughout the incubation period. A slight decrease in counts was occasionally observed after 96 h of incubation.

Twice as much acid was produced in samples containing ginger as in the control (Fig. 1). The amount of acid produced was similar for all three concentrations of ginger tested, and no significant differences in bacterial counts were observed until at least 72 h of fermentation.

Mace stimulated acid production by the starter culture only slightly and the acidity produced at the 4, 8 and 12 g/l levels did not differ significantly (Fig. 2). Bacterial counts for all samples were in the 10^8 cells/ml range after 24 and 48 h and decreased slightly during the later stages of incubation.

Acid production by Lactacel MC starter culture increased with increasing concentrations of mustard

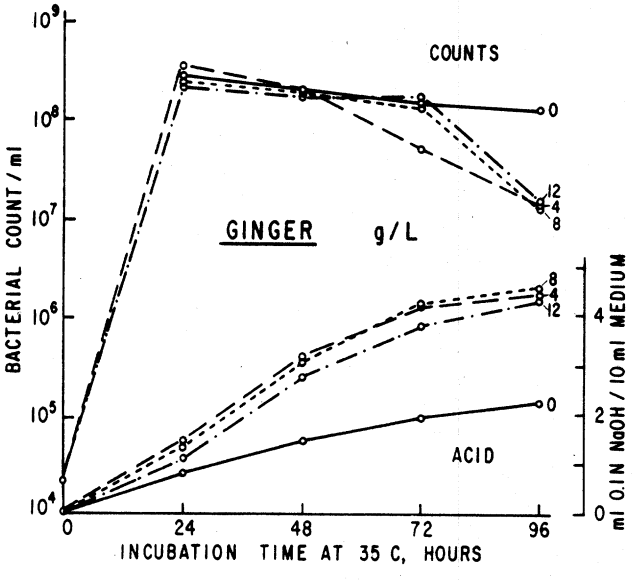

Figure 1. *Effect of 4, 8, and 12 g/l ginger on growth of and acid production by Lactacel MC starter culture organisms in liquid medium.*

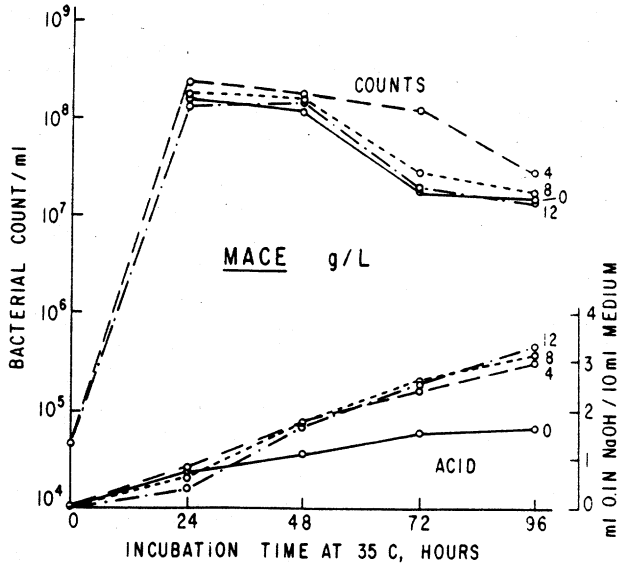

Figure 2. *Effect of 4, 8, and 12 g/l mace on growth of and acid production by Lactacel MC starter culture organisms in liquid medium.*

(Fig. 3). At the 12 g/l concentration, three times as much acid was produced as in the control sample. Bacterial counts for the control and the samples containing 4, 8 and 12 g/l mustard were similar and were in the range 2.5-4.0 × 10^8 cells/ml at 24 and 48 h.

Red pepper (Fig. 4), like mustard, strongly stimulated acid production, particularly in the initial stages of fermentation. Bacterial counts for the control and the samples containing red pepper were practically identical during 4 days of fermentation. Acid production increased with increasing concentration of red pepper at all stages of fermentation. Titratable acidities after 96 h for the control and for samples containing 4, 8 and 12 g/l red pepper were 2.29, 4.15, 5.64 and 6.83 ml, respectively.

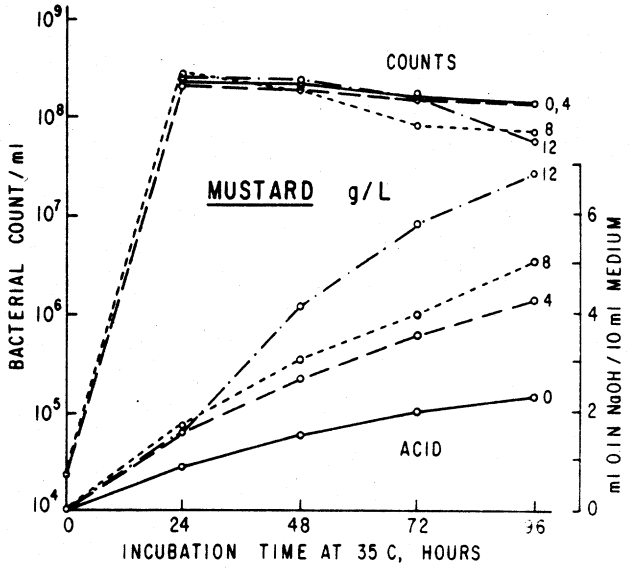

Figure 3. *Effect of 4, 8, and 12 g/l mustard on growth of and acid production by Lactacel MC starter culture organisms in liquid medium.*

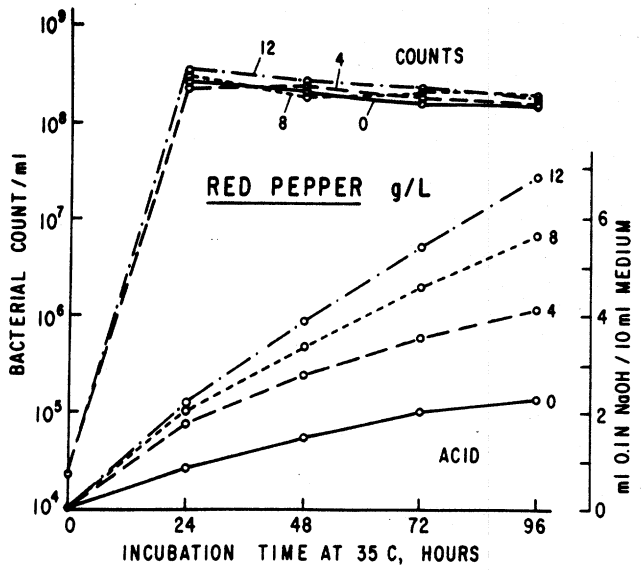

Figure 4. *Effect of 4, 8, and 12 g/l red pepper on growth of and acid production by Lactacel MC starter culture organisms in liquid medium.*

The effect of cinnamon (Fig. 5) on acid production by the starter culture organisms was different from the effects of the above-mentioned spices. Titratable acidities for all cinnamon-containing samples were greater than for the control after 96 h; however, the amount of stimulation diminished with increasing concentration of cinnamon. There was a definite inhibition of acid production in cinnamon-containing samples in the initial stages of fermentation. After 24 h, the sample with 4 g/l cinnamon had an acidity value similar to that of the control, while no acid was formed in the 8 and 12 g/l samples. In fact, at the 12 g/l level, acid production did not take place until after 48 h of fermentation. However, bacterial growth was not inhibited in any of the cinnamon-containing samples at

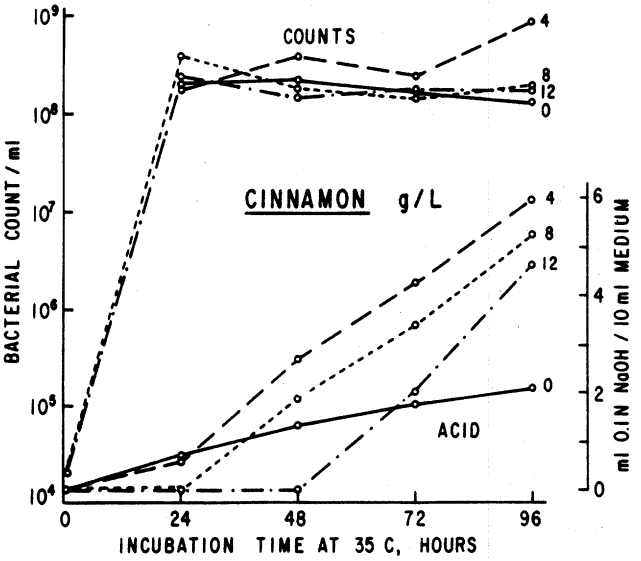

Figure 5. *Effect of 4, 8, and 12 g/l cinnamon on growth of and acid production by Lactacel MC starter culture organisms in liquid medium.*

any stage in the fermentation, and all counts fell into a relatively narrow range around 3×10^8 cells/ml. This is in contrast to the findings of a number of workers that cinnamon possesses strong antimicrobial properties toward some microorganisms (*3,7,10*).

To investigate this effect further, cinnamon was added to the liquid medium in concentrations ranging from 0.5 to 8 g/l. Acid production (Fig. 6) increased with increasing concentration of cinnamon, reaching a maximum at the 4 g/l level, and then declined at the 8 g/l level. In the presence of 2 g/l cinnamon or less, acid production was enhanced even after 24 h, while at higher concentrations acid production was initially inhibited but at later stages enhanced. Bacterial counts (Table 1) for samples containing up to 4 g/l cinnamon were in the same range as the control, 10^8 cells/ml throughout the incubation period. In this instance the sample containing 8 g/l cinnamon had a slightly lower count after 24 h, 2.0×10^7, but the count reached 1.7×10^8 cells/ml after 48 h.

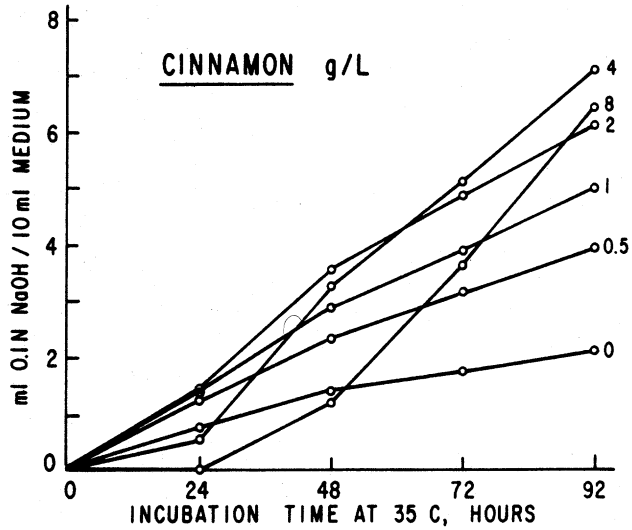

Figure 6. *Effect of 0.5, 1, 2, 4, and 8 g/l cinnamon on acid production by Lactacel MC starter culture organisms in liquid medium.*

Initial experiments indicated that no acid was produced in samples containing 8 g/l clove. This was expected since clove has been reported to show antimicrobial activity toward various species of microorganisms. Therefore the effect of clove on Lactacel MC starter culture was tested at concentrations ranging from 0.5 to 8 g/l. Bacterial counts (Fig. 7) for samples containing 0.5 g/l clove did not differ significantly from those of the control. However, with increasing concentration of clove, increased inhibitory effects on the starter culture organisms were observed. Clove was definitely inhibitory at the 8-g/l concentration. In spite of its inhibitory effect at higher concentrations, clove stimulated acid production by the starter culture bacteria at low concentrations (Fig. 8). In fact, twice as much acid was produced in samples containing 0.5 g/l clove as in the control. At the 2 g/l level, the amount of acid produced was equal to that of the control, even though the bacterial count was about 2 logs less than that for the

TABLE 1. *Effect of cinnamon on growth of starter culture* (L. plantarum *and* P. cerevisiae) *at 35 C.*

Cinnamon (g/l)	Bacterial count[a]/ml			
	24 h	48 h	72 h	96 h
0	1.9×10^8	2.0×10^8	1.2×10^8	8.3×10^7
0.5	2.3×10^8	3.2×10^8	2.4×10^8	2.1×10^8
1	2.4×10^8	3.4×10^8	3.6×10^8	2.2×10^8
2	2.9×10^8	8.8×10^8	9.6×10^8	1.0×10^8
4	1.7×10^8	2.0×10^8	3.2×10^8	7.6×10^7
8	2.0×10^7	1.7×10^8	2.3×10^8	3.0×10^8

[a]The initial bacterial count was 3.9×10^4 cells/ml.

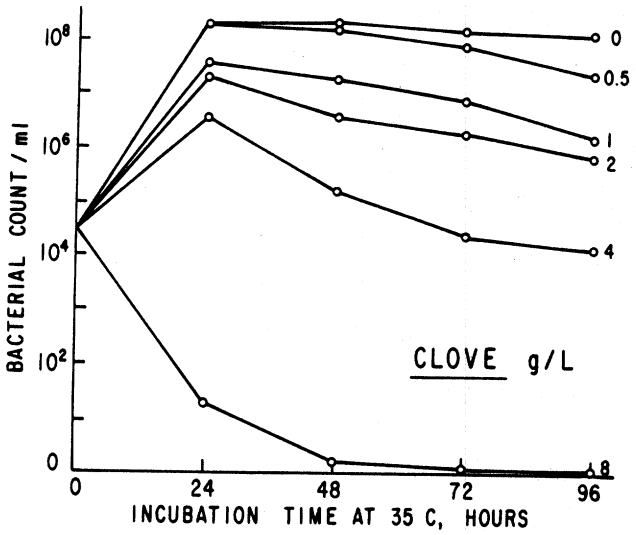

Figure 7. *Effect of 0.5, 1, 2, 4, and 8 g/l clove on survival of Lactacel MC starter culture organisms in liquid medium.*

control. This indicates strong stimulation by clove of acid production by the starter culture bacteria.

The effect of clove at concentrations of 0.5 to 8 g/l was also tested on the individual components of the mixed starter culture, *L. plantarum* and *P. cerevisiae*. Results for the individual microorganisms (Table 2) were similar to those obtained for the mixed culture. Low levels of clove stimulated acid production by both starter organisms, but to a greater extent by *L. plantarum*. Increasing concentrations of clove were inhibitory to both organisms, and 8 g of clove/l was bactericidal.

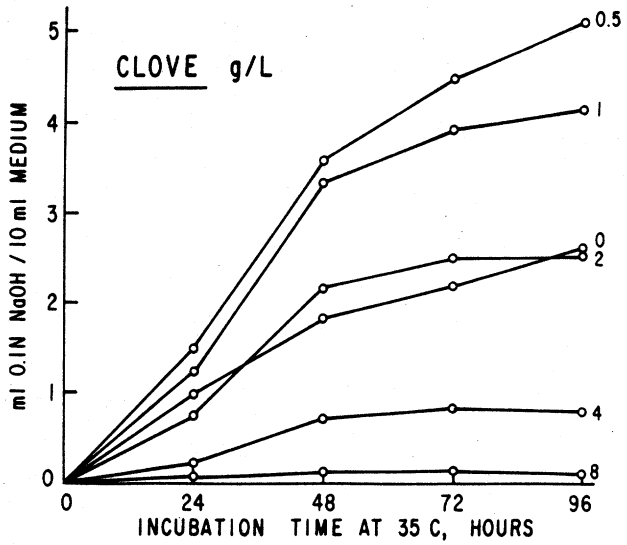

Figure 8. *Effect of 0.5, 1, 2, 4, and 8 g/l clove on acid production by Lactacel MC starter culture organisms in liquid medium.*

Our data suggest that the starter culture organisms, *L. plantarum* and *P. cerevisiae*, are more resistant to the inhibitory effects of spices than are many of the microorganisms investigated by other workers. Of the spices that we studied, cinnamon and clove have been reported to inhibit a variety of yeasts, molds and bacteria, while the other spices were considered inactive or active only at high concentrations (*1,3,5-7,10,21*). Mustard was reported to be particularly inhibitory to yeast (*5,22*). Fabian et al. (*7*) reported that ground cinnamon and clove inhibited pathogenic bacteria at concentrations as low as 0.1%, while mustard, mace and ginger were inhibitory at 5% concentration. Although we found that the growth of Lactacel MC starter culture bacteria was unaffected by red pepper, Gál (*8*) found that capsaicin, the hot principle of red pepper, at a dilution of 1:1000, slightly inhibited growth of *L. plantarum*, did not affect *Staphylococcus aureus* or *Escherichia coli*, but prevented growth of *Bacillus subtilis* and *Bacillus cereus*.

There are only a few reports in the literature dealing with stimulatory effects of spices. Webb and Tanner (*21*)

TABLE 2. *Effect of clove on growth of and acid production by* L. plantarum *and* P. cerevisiae.

Bacterium	g/l	Incubation time at 35 C							
		24 h		48 h		72 h		96 h	
		TA[a]	count/ml	TA	count/ml	TA	count/ml	TA	count/ml
L. plantarum[b]									
Control	—	0.55	4.0×10^7	0.75	1.8×10^7	1.00	1.2×10^7	1.16	8.0×10^6
Clove	0.5	1.12	4.9×10^7	3.62	4.1×10^7	4.96	2.3×10^7	5.33	1.1×10^7
Clove	1	1.00	1.7×10^7	2.73	1.1×10^7	3.39	4.3×10^6	3.55	1.8×10^6
Clove	2	0.61	1.2×10^7	1.64	6.7×10^6	2.00	1.7×10^6	2.16	6.9×10^5
Clove	4	0.40	3.6×10^6	0.69	8.4×10^5	0.78	6.4×10^4	0.78	1.3×10^4
Clove	8	0.14	5	0.12	2.0×10^2	0.15	< 1	0.16	< 1
P. cerevisiae[c]									
Control	—	0.62	1.6×10^8	1.11	9.3×10^7	1.38	3.6×10^7	1.49	2.1×10^7
Clove	0.5	1.12	1.4×10^8	2.59	9.7×10^7	3.33	3.8×10^7	3.71	1.0×10^7
Clove	1	0.85	1.0×10^8	2.60	4.7×10^7	3.08	9.4×10^6	3.27	1.7×10^6
Clove	2	0.19	2.5×10^7	1.18	1.6×10^7	1.77	7.1×10^6	1.99	8.2×10^5
Clove	4	0.09	8.0×10^4	0.18	2.9×10^5	0.30	3.0×10^5	0.44	1.8×10^5
Clove	8	0.14	1.2×10^2	0.22	2	0.24	< 1	0.31	< 1

[a]TA = Titratable acidity.
[b]All samples initially contained 3.4×10^4 cells/ml of Lactacel DS starter culture.
[c]All samples initially contained 1.6×10^4 cells/ml of Lactacel starter culture.

stated that oils of black and white peppers appear to contain growth stimulants for yeasts; however, they did not present data to substantiate this claim. Corran and Edgar (*5*), reporting on the preservative action of spices against yeast fermentation, measured by loss of glucose from the medium, suggested that black pepper contains a yeast stimulant. Salzer et al. (*19*) also reported that black pepper stimulated growth of micrococci, but their findings were inconclusive. Wright et al. (*22*), however, showed that a number of spices at low concentration, including cinnamon, ginger and mace, exhibited marked stimulation of gas production during yeast fermentation and that the enhanced gas production was not the result of accelerated yeast cell proliferation.

Many investigators were concerned only with screening spices and spice components for antimicrobial properties toward a variety of microorganisms. However, our results indicate that although spices may not affect the population of the starter culture bacteria, they may affect production of metabolites by the microorganisms. Also, the concentration at which spices are used is important; large quantities may be germicidal while low concentrations may stimulate some activity of the microorganism. The stimulatory effects we observed might possibly be attributed to trace metals, co-factors, enzymes or other constituents of spices. Additional information is needed to define the mechanism of interaction between spices and microorganisms.

ACKNOWLEDGMENTS

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